Abstract

Background and objectives:

Telomeres are the repetitive DNA sequence found at the ends of eukaryotic chromosomes. These structures play a vital role in maintaining the integrity of the genome and prevent the loss of genetic material that resides near the ends of the chromosome. Telomeres have been considered to be transcriptionally silent, but it was recently demonstrated that mammalian telomeres are transcribed into telomeric repeat-containing RNA (TERRA). TERRA, a long non-coding RNA, participates in the regulation of telomere length and telomerase activity.

Telomerase is a reverse transcriptase capable of adding TTAGGG repeats to the 3' ends of chromosomes. A ribonucleoprotein, the telomerase enzyme is composed of a catalytic protein subunit (telomerase reverse transcriptase, TERT) and an RNA component (telomerase RNA component, TERC). In this thesis, we studied the effect of hTERT repression on the TERRA expression and telomere length in multiple passages.

Methods:

AGS gastric cancer cell line was treated with hTERT FlexiTube siRNA and incubated for 36 hours. Cell viability was examined by MTT assay. A DAPI staining method was used to analysis cell cycle by Flow Cytometry. Real time PCR was used to quantitate the expression level of the human telomerase reverse transcriptase gene (hTERT), TERRA expression and Telomere Length.

Results:

The MTT assay results showed increasing the exposure time to 48 hours decreased cell viability below the 50% viability mark. The flow cytometry cell cycle analysis showed a significant

increased the number of cells in G1 phase and decreased the number of cells in S phase. The results of real-time qRT-PCR analysis demonstrated that downregulation of hTERT expression has no significant effect on TERRA expression in early passages, but siRNA treated cell in the passage of 20 shows a significant change in expression of TERRA compared to these control cell. Also telomere length measurement in each passage was decreased after hTERT siRNA treatment.

Conclusion:

The significant downregulation in hTERT mRNA after 48 hours of hTERT siRNA treatment inhibited the cell viability of AGS cells and cell cycle arrest. This study provides the low expressed of TERRA levels in AGS cell line and downregulation of hTERT expression had no effect on TERRA expression level in early passages of AGS cell line. Also, this study showing a direct link between decreased of telomere length and downregulation of hTERT expression.

Key Words: hTERT, TERRA, AGS, Gastric cancer